

REMARKS

Entry of the above amendment prior to examination is respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims. The attached pages are captioned "**Version with Markings to Show Changes Made.**"

I. Amendments

The specification has been amended in accordance with 37 C.F.R. §1.821 through 1.825 to add the Sequence Listing.

The specification has further been amended to remove embedded hyperlinks in accord with MPEP §608.01.

The specification and claims have been amended in accordance with C.F.R. §1.821(d) to add SEQ ID NO:s.

No new matter is introduced by way of these amendments.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 846-7615.

Respectfully submitted,



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Date: March 22, 2002

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Version with Markings to Show Changes Made

In the specification:

Figure 2 shows the predicted amino acid sequence (SEQ ID NO:2) based on the nucleotide sequence provided in Figure 1 (SEQ ID NO:1).

A Basic BLASTN search ([<http://www.ncbi.nlm.nih.gov/BLAST>) of the non-redundant nucleic acid sequence database was conducted on October 30, 2001, with the *bgl5* gene sequence presented in Figure 1 (SEQ ID NO:1), indicated that the only sequence producing significant alignments (i.e. with an E value of 10^{-5} or less) was GenBank Accession Number AB003109 (*bgl4* gene for beta-glucosidase of *Humicola grisea* var. *thermoidea*; E value 3×10^{-5}).

Figure 2 shows the predicted amino acid sequence (SEQ ID NO:2) of an exemplary BGL5 polypeptide based on the nucleotide sequence provided in Figure 1 (SEQ ID NO:1). The predicted molecular weight of the encoded BGL5 polypeptide is 74.8 kDa. No sequence resembling a signal peptide (Nielsen, H., Engelbrecht, J., Brunak, S., von Heijne, G., Protein Engineering, 10:1-6, 1997) is present at the amino terminus of BGL5 suggesting that the BGL5 polypeptide is not secreted.

A Basic BLASTP search ([<http://www.ncbi.nlm.nih.gov/BLAST>) of the non-redundant protein database, conducted on October 30, 2001 with the BGL5 amino acid sequence indicated 51% sequence identity to GenBank Accession Number AB003109 (beta-glucosidase of *Humicola grisea* var. *thermoidea*), 52% sequence identity to GenBank Accession Number AB003110 (beta-glucosidase of *Hypocrea jecorina*), 47% sequence identity to GenBank Accession Number AF268911 (beta-glucosidase precursor of *Aspergillus niger*), 45% sequence identity to GenBank Accession Number AF149311 (raucaffricine-o-beta-D-glucosidase of *Rauvolfia serpentina*), and 45% sequence identity to GenBank Accession Number AB016877 (beta-glucosidase of *Arabidopsis thaliana*). The ten sequences having highest identity but less than 52% identity with BGL5 were all annotated as beta-glucosidases. These sequence similarities indicate that BGL5 is a member of glycosyl hydrolase family 1 (Henrissat, B. and Bairoch, A. (1993) Biochem. J. 293:781-788).

Preferred culture conditions for a given filamentous fungus may be found in the scientific literature and/or from the source of the fungi such as the American Type Culture Collection (ATCC;

"[http://]www.atcc.org/"). After fungal growth has been established, the cells are exposed to conditions effective to cause or permit the over expression of BGL5.

Exemplary computer programs which can be used to determine identity between two sequences include, but are not limited to, the suite of BLAST programs, *e.g.*, BLASTN, BLASTX, and TBLASTX, BLASTP and TBLASTN, publicly available on the Internet at [http://]www.ncbi.nlm.nih.gov/BLAST/. See also, Altschul, *et al.*, 1990 and Altschul, *et al.*, 1997.

The *T. reesei* RNA is used as template for RT-PCR using methods known in the art (Loftus, J. et al., Science, 249:915-918, 1990). During this procedure the mRNA is reverse transcribed to produce first strand cDNA. The cDNA subsequently serves as template for PCR amplification of *bgl5* cDNA sequences using specific oligonucleotide primers designed in accordance with SEQ ID No. 1 or SEQ ID No. [4]3.

In the claims:

2. An isolated polynucleotide selected from the group consisting of:

(a) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL5 polypeptide having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);

(b) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL5 polypeptide having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);

(c) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL5 polypeptide having at least 95% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);

(d) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL5 polypeptide having the amino acid sequence presented in Figure 2 (SEQ ID NO:2);

(e) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL5 polypeptide having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;

(f) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL5 polypeptide having the amino acid sequence presented as SEQ ID NO:2;

(g) a nucleic acid sequence presented as SEQ ID NO:3, or the complement thereof;
and

(h) a nucleic acid sequence that hybridizes, under high stringency conditions to the sequence presented as SEQ ID NO:3, or the complement or a fragment thereof, wherein said isolated polynucleotide encodes a polypeptide having the biological activity of a β -glucosidase

8. An expression construct including a polynucleotide sequence (i) having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2), or (ii) being capable of hybridizing to a probe derived from the nucleotide sequence disclosed in Figure [2]1 (SEQ ID NO:1) under conditions of intermediate to high stringency, or (iii) being complementary to a nucleotide sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2).

18. A substantially purified BGL5 polypeptide with the biological activity of a β -glucosidase, comprising a sequence selected from the group consisting of:

(a) an amino acid sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);

(b) an amino acid sequence having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);

(c) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);

(d) an amino acid sequence presented in Figure 2 (SEQ ID NO:2);

(e) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;

(f) an amino acid sequence presented as SEQ ID NO:2;

(g) a substantially purified biologically active fragment of the amino acid sequence presented as SEQ ID NO:2.

25. A detergent composition, said composition comprising a polypeptide selected from the group consisting of:

(a) an amino acid sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);

(b) an amino acid sequence having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);

(c) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);

(d) an amino acid sequence presented in Figure 2 (SEQ ID NO:2);

(e) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;

(f) an amino acid sequence presented as SEQ ID NO:2;

(g) a substantially purified biologically active fragment of the amino acid sequence presented as SEQ ID NO:2.